

REMARKS

Claims 1 and 3-18 are pending in the application. Claims 1, 4-9 and 11-18 have been amended. Claims 20-22 have been added.

Claims 1, 4-9, and 11-18 are amended to clarify the claim language. Claims 1, 4, and 7 are also amended to remove the phrase “ingredients equivalent to the conditioned medium”. Claims 17 and 18 are amended to recite a pharmacologically acceptable carrier. Support for the amendments to claims 17 and 18 can be found in the Specification at page 35, lines 22-23.

New claim 20 is added. Support for claim 20 can be found on page 17, lines 23-24 and page 19, lines 1-5. New claim 21 is added. Support for new claim 21 is found in the Specification at page 39, lines 1-8. Support for new claim 22 is found in the Specification at page 5, line 23 and in Figure 23.

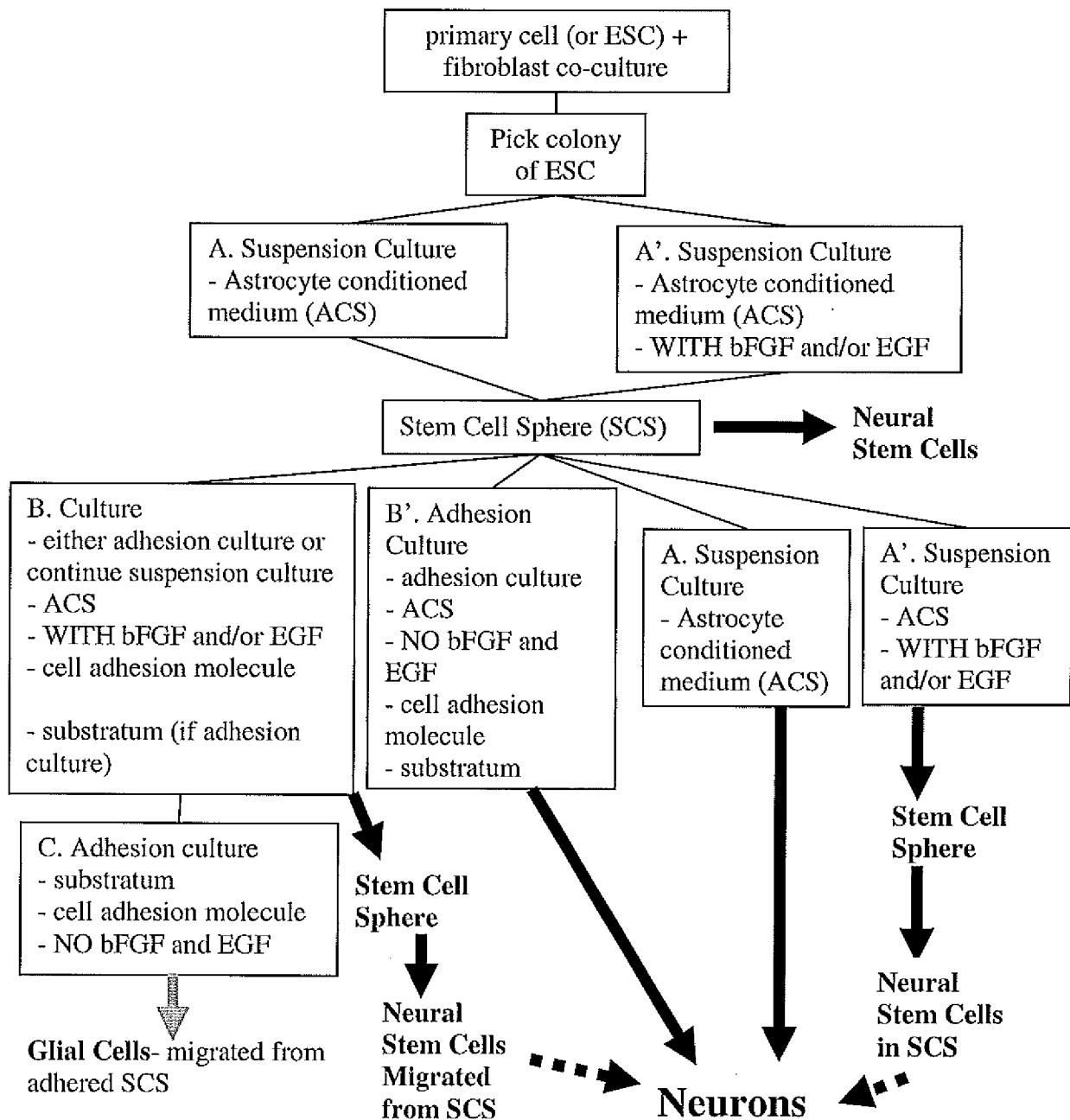
Applicants respectfully submit that no new matter has been added and request entry of the amendments.

1. Rejections under 35 U.S.C. §103(a)

The Examiner rejects claims 1, 3-4, 8-12, and 13-18 under 35 U.S.C. § 103(a) as being unpatentable over Tropepe, Weiss, and Suemori. The Examiner also rejects claims 5-7 under 35 U.S.C. § 103(a) as being unpatentable over Tropepe, and Weiss, in view of Vitokovic, Reubinoff, and Thompson. These rejections are respectfully traversed.

The claimed invention relates to a method for producing isolated neural cells. The method includes a first method of culturing embryonic stem cells in the presence of astrocyte conditioned medium, which produces Stem Cell Spheres and directly produces isolated neural cells. The method may further comprise a second culturing step, which may include bFGF or EGF and a cell adhesion molecule, and it may include a substratum. These methods, depending on whether they are adherent culture, or whether the growth factors are present, generate neural stem cells (within or as migrated from the Stem Cell Sphere), neurons, or if continued, glial cells.

The method of the present invention can be diagrammed as follows: (note dashed lines indicate a possible outcome)



A. Tropepe, Weiss and Suemori

- a. The Examiner has not provided a reason why one of skill in the art would choose the particular conditions from the references.**

Applicants submit that the Examiner fails to establish a *prima facie* case of obviousness from the cited references. In particular, the Examiner has impermissibly used hindsight to make the instant rejection, utilizing the claims as a "template" upon which to assemble references, each reciting an element of the claim. Such an approach is impermissible.

Such an approach to making an obviousness rejection has been repeatedly rebuked by the courts. See, e.g. *Sensonics, Inc. v. Aerosonic Corp.*, 38 USPQ2d 1551, 1554 (Fed. Cir. 1996):

To draw on hindsight knowledge of the patented invention, when the prior art does not contain or suggest that knowledge, is to use the invention as a template for its own reconstruction -- an illogical and inappropriate process by which to determine patentability. *W.L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed.Cir. 1983). The invention must be viewed not after the blueprint has been drawn by the inventor, but as it would have been perceived in the state of the art that existed at the time the invention was made. *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1138, 227 USPQ 543, 547 (Fed.Cir. 1985).

See also, *In re Omeprazole Patent Litigation*, 82 USPQ2d 1643, 1656 (Fed. Cir. 2007).

The Examiner cannot properly establish *prima facie* obviousness by merely listing a set of references that together set forth each of the elements of the claim individually. A claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. *KSR Int'l Co. v. Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007). There must be a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does. *Id.*

Furthermore, the Examiner is not permitted to merely extract from cited references those teachings that support a conclusion of obviousness. Rather, the references must be considered as a whole. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 220 USPQ 303 (Fed. Cir. 1983):

In its consideration of the prior art, however, the district court erred in ...considering the references in less than their entirety, i.e., in disregarding disclosures in the references

that diverge from and teach away from the invention at hand. *In re Kuderna*, 426 F.2d 385, 165 USPQ 575 (CCPA 1970).

That the Examiner has used a "templating" approach to making the instant rejection is apparent from the disparate disclosures used as references. In particular, the Examiner equates disparate cell populations (in both age and type), disparate culture techniques, varying culture media, and simply assumes that a technique or ingredient which is applicable to one cell population is equivalent to applying that technique or ingredient to another cell population.

For instance, the Examiner states that "Weiss teaches a method of producing a neuron by carrying [out] a suspension of embryonic stem cells in the presence of ingredients substantially equivalent to an astrocyte conditioned medium." (Office Action, December 10, 2009, page 3, citing Example 8 of Weiss, relied on in the Office Action of June 10, 2010, page 2).

However, Example 8 of Weiss begins with neurospheres produced in Example 3 (Weiss, col. 15, lines 12-13). Example 3 begins with primary cells excised from mouse embryo brain tissue. The primary cells are suspended in a complete medium and plated (adherent culture) (Weiss, col. 12, Example 3, beginning at line 11). After 3-4 days, the cells "formed neurospheres that lifted off the substrate." (Weiss, col. 12, lines 24-25).

Thus, by the time the cells are placed in an astrocyte conditioned medium, they have already formed neurospheres. At that point the cells are NO longer merely embryonic stem cells, but are instead defined by Weiss to be a precursor cell. (Weiss, col. 8, line 62-65). In fact, Weiss defines "precursor cells" to be "the progeny of neural stem cells, and thus include[] both progenitor cells and daughter neural stem cells." (Weiss, col. 8, line 59-62). Weiss describes that these "precursor cells" in a cell cluster "are immunoreactive for nestin." (Weiss, col. 9, lines 26-27). Nestin is a well known marker for neural stem cells, and undifferentiated embryonic stem cells are NOT reactive for nestin (See Specification, Figure 23, col. 1, page 5, line 23, and page 44, line 14). Thus, the embryonic stem cells of the instant claims are not equivalent to the precursor cells of Example 8 of Weiss, and are certainly not the progeny of neural stem cells.

Therefore, in Weiss, ACS is used only in later culture steps upon cells which are already the progeny of neural stem cells. In addition, Applicants maintain that while the claims no longer recite the suspension culture of ESCs in “ingredients equivalent to” astrocyte conditioned medium, such a method is within the scope of the disclosure presented in the present application.

Like Weiss, Tropepe takes a commercially available ESC line, but again cultures them initially with DMEM, FCS and LIF. (Tropepe, page 75, left col., lines 19-21)¹ The culture for neurosphere production is described as a “chemically defined serum-free media” with LIF and/or EGF or FGF2. (Tropepe, page 75, lines 24-28). Again, even in combination with Weiss, there is no mention of astrocyte conditioned medium for the culture step of embryonic stem cells. The Examiner has failed to explain why one of skill would be led to apply a drastically different (and not recommended Weiss col. 7, lines 12-14) type of medium to the ESC described in Tropepe.

It is also clear that the Examiner is using hindsight because the Examiner has ignored that Tropepe describes the frequency of colony formation in the presence of LIF as pitifully low. (Tropepe, page 66, right col., lines 17-19, reciting 0.2% sphere colony formation). Such a teaching would disincentivize one of skill in the art from pursuing this type of method if the goal was to obtain a large number of neural stem cells.

The Examiner has attempted to justify why these teachings in Weiss should not be considered by referring to Tropepe, however, her reasoning fails to reasonably explain why LIF might be considered equivalent to astrocyte conditioned media in the first culture step. The Examiner alleges that “Weiss is not cited for the source of embryonic stem cells . . . but Weiss is cited to supplement the teachings of Tropepe for culturing the SCS obtained from Tropepe (step A) in the state of adhesion of SCS” in the absence of bFGF and in the presence of ACS or ingredients equivalent to the conditioned medium.” (Office Action, June 10, 2010, pages 6-7). The Examiner presumably then relies on Tropepe and Suemori alone to provide the teachings for Step A that is, carrying out the suspension culture of embryonic stem cells in the presence of the

¹ In fact, Tropepe refers to the methods of the Weiss lab on page 66, col. 1, lines 10-16, when discussing the adhesion culture of ESCs to form neurospheres.

astrocyte conditioned medium or ingredients equivalent to the conditioned medium, thereby forming a stem cell sphere (SCS). The Examiner appears to allege that since culture with a defined medium and LIF obtains an ES-derived sphere *colony*, it is the equivalent to culture with ACM to obtain a Stem Cell Sphere. (Office Action, June 10, 2010, page 5)²

However, the sphere colony (aka, “colony”, “colony-forming ES-cells”, “ES-derived sphere colony”) is again **NOT EQUIVALENT** to embryonic stem cells, in that it has already undergone some differentiation (See also, Tropepe, page 67, lines 15-30, and figure 1(c), expressly indicating that sphere *colonies* express nestin, and that “Nestin expression is correlated with the initial formation of the sphere colony”).

Even further it is clearly not the case that LIF alone is “equivalent” to ACM, that is, that culture with LIF would be sufficient to obtain a neural stem cell (either by the Stem Cell Sphere method of the present application, or by the embryoid body method of Tropepe and Weiss). Both Applicants and Tropepe use an LIF-containing medium to *proliferate* the ESC. (Specification, page 39, lines 1-8; Tropepe, page 75, lines 19-22). Thus, if the presence of LIF alone were sufficient to obtain either a stem cell sphere or an ES-derived sphere colony, the scientists of the present application would have seen the generation of these bodies during ESC *proliferation*.

The Specification defines “ingredients substantially equivalent to the conditioned medium” as “ingredients which are capable of exhibiting the same action as that of the conditioned medium and refers to ingredients obtained by removing the ingredients of the basal medium used and the astrocytes from the culture of astrocytes, for instance, metabolites and such.” (Specification, page 19, lines 1-5). Thus, as discussed above, LIF does not exhibit the same action as ACM. The Specification defines, however, what is considered equivalent to ACM in terms of the specific ingredients, none of which are taught in Tropepe, Weiss, and Suemori. Consequently, Applicants submit that the Examiner’s interpretation that “equivalent” to ACM includes LIF is

² Applicants note that the claim language no longer includes ingredients equivalent to ACM for claim 1, step A or A’. Applicants maintain that even with the “ingredients equivalent” language, the combination of Tropepe, Weiss and Suemori do not teach claim 1 or parts A or A’. Because the combination of Tropepe, Weiss and Suemori do not teach suspension culture of ESCs in ACM, Applicants request that the rejection be withdrawn.

factually incorrect and legally insufficient, and maintain that the claims may encompass “ingredients equivalent to the conditioned medium”.

Furthermore, Tropepe, like Weiss, “plates” the proliferated embryonic stem cells in wells precoated with poly-L-ornithine to obtain the ES-derived sphere colonies. (Tropepe, page 75 right column, line 31). Poly-L-ornithine is used in the art of tissue culture to *assist* with the adherence of cells to the plate or flask. Therefore, like Weiss, the culture of ESCs themselves is an adherent step, not a suspension culture. This is further evidence that the Examiner has taken the methods used in a second culture step and applied them piecemeal to the initial culture of embryonic stem cells.

Suemori is cited only for the “deficiency of culturing primate embryonic stem cells and not for the stage of differentiation of the SCS.” (Office Action, page 9) Applicants do not dispute that Suemori teaches the culture of primate embryonic stem cells and the cryopreservation of the cells resulting from those cultures. However, as discussed above, Applicants submit that the combined references do not teach the suspension culture of embryonic stem cells in an astrocyte conditioned medium. As the Examiner has conceded that the Suemori reference does not speak to this element, Applicants submit that the addition of Suemori to Tropepe and Weiss does not remedy the deficiencies of Tropepe and Weiss.

In summary, the Examiner has impermissibly taken the culture conditions applicable to adherent cultures of ESCs, and combined them with the suspension culture conditions applicable to neurospheres, and impermissibly ignored the teachings in both Weiss and Tropepe which highlight the differences in the methods. The Examiner has not provided a reasonable explanation as to why one of skill in the art would modify the methods and the cell populations in order to obtain the claimed method. Accordingly, Applicants respectfully submit that the Examiner has impermissibly used hindsight to attempt to establish a *prima facie* case of obviousness. Applicants maintain that because the Examiner has failed to provide a reasonable explanation for the modifications, the combination of Tropepe, Weiss, and Suemori fail to

establish that the claimed methods would have been obvious to one of skill in the art at the time of filing. Applicants request that the rejection be withdrawn.

b. The presently claimed invention presents unexpected results.

Applicants submit that even if the Examiner had successfully established a *prima facie* case of obviousness, the results of the presently claimed method are unexpected. The presently claimed methods generate stem cell spheres, and an isolated population of desirable cells in large numbers.

Specifically, the present method generates a large number of neural stem cells from the total population of embryonic stem cells, in a short period of time, which can then produce an isolated population of a specific type of neuron (e.g., dopaminergic neuron, GABAergic neuron, or a cholinergic neuron), or glial cells. (Specification, page 3, lines 7-8, page 15, lines 5-12, 18-20). In a stem cell sphere, by day 4 of suspension culture, 100% of the cells express nestin (See Specification, Figure 24, described at page 14, lines 17-22, and page 62, lines 8-16, describing the amount of mRNA for nestin, which is expressed in neural stem cells and neurons, divided by the amount of GAPDH, which is expressed by all cells). By day 2 of adhesion culture after suspension culture (step B and B'), all of the cells also expressed TH (an indicator of a differentiated neuron). (*Id.*)

In contrast, the method of Tropepe demonstrates that of the number of ESC used, only 0.2% or 0.3% of those cells ever form an ES-derived sphere and become nestin positive. (Tropepe, page 66, right col., lines 18-19, page 67, left col., lines 4-5 and left col., lines 15-34).

Directly comparing the two, it is clear that the present invention generates more than 333-fold increase in the number of neural cells when compared to the closest prior art reference. Applicants submit that this overwhelming increase in neural cells would have been unexpected to one of skill in the art at the time of filing. Also, Applicants submit that such an overwhelming recovery of isolated neural cells is sufficiently unexpected to overcome any case of *prima facie* obviousness that may have been established. Applicants request that the rejection be withdrawn.

B. Tropepe, Weiss, Vitkovic, Reubinoff, and Thompson

The Examiner also rejects claims 5-7 under 35 U.S.C. § 103 as being unpatentable over Tropepe, Weiss, Vitkovic, Reubinoff, and Thomson. The deficiencies of Tropepe and Weiss are discussed above. However, the combination of Vitkovic, Reubinoff, and Thompson does not remedy these deficiencies.

The Examiner states that “the combination of Vitkovic, Reubinoff, and Thompson is cited to cure the deficiency of step (B) which requires the presence of bFGF. Vitkovic is cited for the use of culture medium containing bFGF and not for the presence of other growth factors of serum as applicants assert.” (Office Action, page 12)

Since the Examiner asserts that the combination of Vitkovic, Reubinoff and Thomson are not used for step A (A' or claim 1), Applicants maintain that the combination of Tropepe and Weiss fail to teach this step, and thus, the combination fails to teach claims 5-7 (which all require step A (A' or claim 1).

Moreover, Applicants submit that the Examiner again reveals that she is impermissibly using hindsight to pick and choose elements from disparate methods without an explanation of why one of skill in the art would have been led to choose that element from that particular method. In particular, when the Examiner asserts that Vitkovic is cited only for the presence of bFGF, the Examiner provides no explanation as to why one of skill in the art would take the bFGF from the methods of Vitkovic, yet at the same time exclude the serum or other growth factors.

Using the Examiner's own rationale, that “Suemori teaches shared many [sic] characteristics between human ES cells and the cynomolgus monkey, as well as the rhesus monkey, which are closely related to humans then it is obvious for one of skill in the art to use bFGF under the culture conditions of Tropepe/Weiss since the instant claims require no timeline of adding the bGF [sic] . . .” (Office Action, page 12). The Examiner's rationale provides no particular reason

for adding bFGF to step B, and presumably, one of skill in the art would have no guidance in taking any particular ingredients from any particular method of culturing primate embryonic stem cells. Thus, one of skill in the art would also be expected to include the serum from Vitkovic among other ingredients.

Reubinoff is used to “supplement” Vitkovic, and for teaching “the use of bFGF inhuman [sic] ES cells and moreover, [] Thomson teach that directing differentiation of ES cells to specific cell types for therapeutic use.” (Office Action, page 12)

However, none of these references teach step A (A’ or claim 1) from which claims 5-7 depend, and none of them provide a reason to significantly alter the media of Vitkovic by omitting serum. Thus, Applicants maintain that the Examiner has failed to establish a *prima facie* case of obviousness for claims 5-7.

Moreover, the references do not raise the expectation that modifying the method of Tropepe would lead to a higher yield of an isolated population of neural cells. Thus, the unexpectedness of the results of the claimed method apply equally to claims 5-7. For this additional reason, Applicants request that the rejection be withdrawn.

C. Applicants do not argue each reference individually, but instead argue the references as a combination.

The Examiner asserts that “Applicants argue each reference individually. . . . [O]ne cannot show nonobviousness by attacking the references individually where the rejections are based on combinations of references” (Office Action, page 2, citing *In re Keller*, 642 F.2d 413, 208 U.S.P.Q. 971 (CCPA 1981)). Applicants do not disagree with the well-established principles set forth by *In re Keller*.

However, to address the teachings of the prior art in any kind of logical and orderly fashion, the teachings of the references must be addressed. Thus, the deficiencies of the Weiss and Tropepe references were discussed in relation to their failure to teach the suspension culture of embryonic

stem cells in an astrocyte conditioned medium. Since both references fail to teach this recitation, Applicants systematically addressed each of the Examiner's points. That they were in separate sections does not minimize the fact that Tropepe, the primary reference, does not disclose the suspension culture of embryonic stem cells in astrocyte conditioned media and that none of the other references provide this teaching or a reason to perform a suspension culture of ESCs in astrocyte conditioned media.

The emphasis on the fundamental differences in the methods between the claimed methods and the methods of Tropepe, Weiss, and Suemori only highlights that one of skill would have no reason to modify those methods to obtain the claimed method, because they all pertain to culturing and obtaining a different cell population, which expresses different cell markers.

Moreover, with regard to the rejection based on Tropepe, Weiss, Vitkovic, Reubinoff, and Thomson, the Examiner states on page 10 of the Office Action that Applicants are arguing the references individually, but then quotes Applicants' previously response stating "Accordingly, one of skill in the art would have no reasonable expectation of success in combining the fundamentally different methods of Tropepe and Weiss with the significantly different medium of Vitkovic to obtain the cell population obtained by the claimed method." (Office Action, page 11). Thus, the assertion that the references are merely argued individually does not appear to be true. Applicants submit that the Examiner has not made a *prima facie* case of obviousness based on Tropepe, Weiss, Vitkovic, Reubinoff and Thomson.

Applicants therefore request that the rejection be withdrawn.

In view of the foregoing, Applicants believe the pending application is in condition for allowance. A Notice of Allowance is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mary M.H. Eliason, Reg. No. 58,303 at (858) 792-8855, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

By  _____

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Attachments: Replacement Figure 24